Allozymic variability in European anchovy *Engraulis encrasicolus* (L.) along the Moroccan coasts

by

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Abstract. – Allozyme variation was used to investigate for the first time the genetic structure of European anchovies, *Engraulis encrasicolus* (Linnaeus, 1758), along the Moroccan coasts. A total of 420 individuals were collected at 11 sites during the anchovy spawning period of 2012. Eleven loci were tested, among them only *LDH** [E.C:1.1.27] and *PGM* * [E.C:5.4.2.1] were polymorphic. Statistical analysis results showed no significant differentiation between samples (overall F_{ST} = 0.0077, p = 0.13). Genotypic proportions were not in accordance with Hardy-Weinberg predictions; an important heterozygote deficiency was detected (F_{IS} = 0.48959 ± 0.07863, p < 0.001). The results are compared with those of other studies and are discussed in relation to hydroclimatic factors in the Moroccan coasts and to species behaviour in such environment.

Received: 30 Sep. 2014
Accepted: 28 Jan. 2016
Editor: A. Gilles

Key words
Engraulidae
Engraulis encrasicolus
Moroccan coast
Genetic variability
Allozyme variation

Résumé. – Étude de la variabilité allozymique chez l'anchois européen *Engraulis encrasicolus* (L.) le long des côtes marocaines.

L'étude des marqueurs allozymiques chez l'anchois européen, *Engraulis encrasicolus* (Linnaeus, 1758), a porté sur onze échantillons, soit un total de 420 individus adultes, collectés le long des côtes marocaines durant la période de ponte de 2012. Parmi les onze systèmes enzymatiques testés, seules la *LDH** [CE: 1.1.27] et *PGM* * [CE: 5.4.2.2] ont présenté un polymorphisme. L'analyse statistique des données a montré que les échantillons ne sont pas en équilibre de Hardy-Weinberg et présentent un important déficit en hétérozygotes (F_{IS} globale = 0,48959 ± 0,07863, p < 0,001) avec une différenciation génétique non significative entre les échantillons (F_{ST} globale = 0,0077, p = 0,13). Ces résultats sont comparés à ceux qui ont été obtenus dans des études précédentes sur la même espèce et sont discutés par rapport aux facteurs hydroclimatiques relevés au niveau des côtes marocaines et au comportement de l'espèce dans un tel environnement.

The small pelagic fish are one of the most important fish resources in the Northwest African coast. These resources are shared by different countries and are exploited by artisanal and industrial fleets. Despite their importance, like any other fish, these are exposed to reduction of their biomass, which can cause stock collapse and extinction of the species if heavy fishing pressure is maintained (Ferguson, 1995). This concern has raised questions about reduction in the genetic resources of natural fish populations and their safeguard. This has become a global preoccupation. For this reason, molecular genetics research should be strongly supported (particularly: species genetic structuring and stock boundaries), for the long-term management of fisheries resources (Park and Moran, 1995).

The European anchovy, *Engraulis encrasicolus* (Linnaeus, 1758), is a clupeid fish distributed in the Mediterranean, Black and Azov Seas and along the eastern Atlantic coastline from Norway to Angola (Whitehead *et al*., 1988), and it is also present around the tip of Southern Africa (Grant and Bowen, 1998; Kristoffersen and Magoulas, 2008). These fish are coastal schooling planktivores that spawn in batches and grow up to 10-20 cm (Blaxter and Hunter, 1982). Anchovies support a wide range of temperatures (2-30°C) and salinities (5-41 psu) (Whitehead *et al*., 1988).

Due to its abundance, anchovy, like other small pelagic fish, plays a key role in upwelling ecosystems and is characterized as 'wasp-waist' (Rice, 1995; Cury *et al*., 2000). They exercise both top-down and bottom-up control on food webs (Cury *et al*., 2000), since they constitute the intermediate link in the flow of energy from lower to higher trophic levels. The small pelagic fish in such systems are called forage fish because they contribute substantially to fishery catches (Haii, 1999; Duarte, 2004) and to diet of predators. In addition to its ecological importance, anchovies play a major socio-economic role in all regions where this species is one of the principal target for commercial fisheries. For those reasons, a large number of genetic studies have been conducted on anchovies in adjacent regions of Moroccan coast.

Firstly, genetic stock structure of this species was examined by Bembo *et al*. (1996a), using allozymic markers in the northern Mediterranean Sea. They showed a low overall differentiation between analysed samples. Tudela *et al*.

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(1999) examined six samples in different areas between the south of Catalonia (Spain) and the island of Elba (Italy), and found considerable genetic homogeneity. However, Bembo *et al*. (1996b) identified offshore and inshore stocks in the Adriatic, based on electrophoretic and morphological differences that Borsa (2002) possibly equated to two biological species present elsewhere in the Mediterranean Sea. Borsa *et al*. (2004) reported that highly significant θ values were observed in all comparisons between oceanic anchovies and inshore anchovies, while no significant differences were detected among samples within either oceanic or inshore anchovies. They suggested the name of *Engraulis albidus* for lagoonal anchovies in the Gulf of Lions. Sanz *et al*. (2008) used allozymes to study populations on both sides of the Breton Canyon Cape in the Bay of Biscay at the microgeographic scale, and concluded that only one genetic unit (stock) existed in this area. Magoulas *et al*. (1996, 2006), through mitochondrial DNA (mtDNA), reported a significant phylogeographic structure in both the Atlantic and Mediterranean populations of anchovy. They reported two haplotype clades (A and B) separated by 3.2% sequence divergence. However, the recent studies of Borrell *et al*. (2012) (using microsatellite and mtDNA markers), Zarraonaindia *et al*. (2012) (nuclear and mitochondrial SNPs markers) and Viñas *et al*. (2013) (mt DNA markers only) did not sampled purposely coastal forms, although they all recovered some level of genetic differentiation that they attributed to various causes. Zarraonaindia *et al*. (2012), for instance, propose the existence of two groups of anchovies. One group would be associated with areas of deep-water upwelling on narrow continental shelves, such as that of the Iberian Atlantic; the second group associated with wide continental shelves, which would include the NE Atlantic (comprising the North Sea and the Bay of Biscay) and the Mediterranean Sea (reviewed in Oueslati *et al*., 2014). Oueslati *et al*. (2014), by analysing mtDNA and six microsatellites loci, reported that their findings strengthened and extended previous reports of the existence of a coastal anchovy entity that is widespread in Mediterranean lagoons and that is distinct from the more offshore marine populations. Finally, results of Silva *et al*. (2014) on samples from the western Atlantic, eastern Atlantic (from Norway to Ghana) and western Mediterranean Sea, reported that two mitochondrial clades were recovered, consistent with previous studies of *E. encrasicolus*, in which the frequency of each clade varied by latitude. Four genetic clusters, corresponding loosely to large geographical regions, were identified with microsatellite data.

On the Moroccan shelf, annual purse-seine catches exceeded 34,000 tons throughout 2012 (Office national des pêches (ONP), 2013). Despite their importance, little is known about stocks and populations dynamics of this species in this region, because few studies have dealt with anchovy from Moroccan coasts. In order to ensure sustainable catches, exploitation of anchovy necessitates a rational management. This requires a more accurate information on stock boundaries and realistic definition of fishery management units, which reflects biological self-sustaining populations. The focus of the present work is to investigate stock structure of anchovy populations by using allozymic markers along the Moroccan coasts and to investigate hypotheses explaining the observed genetic population structure.

MATERIAL AND METHODS

Sampling

The analysis was conducted in eleven samples of anchovy composed of 30 to 50 individuals each. A total of 420 fish were analysed. They were collected throughout the Atlantic and Mediterranean Moroccan coastlines on board Moroccan research vessel R/V *Amir Moulay Abdallah* during spawning grounds in 2012 (Fig. 1, Tab. I). Fish were dissected to isolate the liver and a piece of muscle. Each piece of tissue was ground manually on crushed ice in 1 ml of distilled water to limit the degradation of enzymatic activity. Homogenates were subdivided into four Eppendorf tubes and stored at -30°C.

Allozyme electrophoresis

In all, eleven enzymatic systems, using three buffers (TC 8.0, TCB 8.7 and TG 9.0), were screened: *LDH**

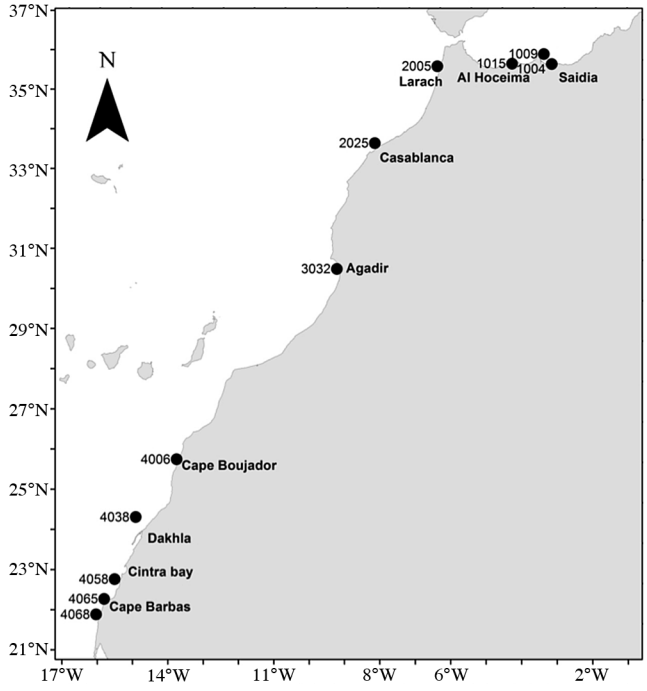

Figure 1. - Map of Moroccan coasts showing the locations of anchovy sampling.

[E.C:1.1.27], *PGM** [EC:5.4.2.1], *SOD** [EC:1.15.1.1], *GPD** [EC:1.1.1.8], *GPI** [EC:5.3.1.9], *IDH** [EC:1.1.1.42], *MDH** [EC:1.1.1.37], *MPI** [EC:5.3.1.8], *ME** [EC:1.1.1.39 or 38], *AAT** [EC 2.6.1.1] and *GAPD** [1.2.1.12]. These were separated on horizontal starch gel electrophoresis following the technique of Pasteur *et al*. (1987) using TG 9 buffer. Alleles and genotypes were scored according to Shaklee *et al*. (1990), the most common allele in the first sample being designated as 100. The other variants were subsequently numbered according to their electrophoretic mobility relative to the most common allele.

Genetic data analysis

Allele and genotype frequencies were obtained by counting phenotypes directly from the gels and analysed with the GENETIX 4.05.2 software (Belkhir *et al*., 2004) (http://www.univ-montp2.fr/genome-pop/genetix.htm). Departure from Hardy-Weinberg proportions was assessed with the Wright's fixation index (F_{IS}) and statistically tested using the Markov chain method implemented in Genepop 4.1.4 (Raymond and Rousset, 1995; Rousset, 2008). Significance levels for statistical tests were adjusted according to the standard Bonferroni procedure (Rice, 1989). The genetic divergence among samples was assessed using the Wright's standardized variance in allele frequencies (F_{ST}) (Wright, 1969). This index was computed over all samples, following the Weir and Cockerham (1984) algorithm available on GENETIX. To calculate genetic distances, a non-biased algorithm was used (Nei, 1978), which was more appropriate for small sample sizes than the standard distance algorithm Ds (Nei, 1972). A phenogram was constructed from genetic distances using the UPGMA method in the software MEGA 6.06 (Tamura *et al*., 2013), via neighbour-joining function implemented in this software, and tested using the hierarchical analysis of molecular variance (AMOVA) implemented in ARLEQUIN Version3.1 (Excoffier *et al*., 2005). The AMOVA provided estimates of the portion of the observed total variance accounted for within and among groups of samples, the objective was to test for the best grouping by maximizing the variance among groups, while minimizing the variance within groups. Statistical significance was determined using a permutation of genotypes among groups (> 1000 permutations). To verify whether the polymorphic loci (*LDH** and *PGM**) are under selection, a Beaumont and Nichols neutrality test (Beaumont and Nichols, 1996) was applied using the LOSITAN software (Antao *et al*., 2008), a method based on the distribution of F_{ST} conditional on gene diversity, rather than allele frequency. They generated, by simulation, the 95% confidence intervals for F_{ST} under distinct genetic models, the estimates of F_{ST} at polymorphic loci were then plotted against their expected gene diversities. Outlier loci were considered under selective regime (Guinand *et al*., 2004).

RESULTS

Among loci, only *LDH** and *PGM** were polymorphic. Statistical analyses showed that all populations are deviated from Hardy-Weinberg equilibrium, which is expressed by a significant deficit in heterozygotes for two analysed loci (overall $F_{IS} = 0.48959 \pm 0.07863$, $p < 0.001$), except for the population of Dakhla (4038) located in the south of Morocco. F_{IS} per population ranged from 0.265 at Dakhla in the south of Moroccan Atlantic to 0.805 at Saidia (1004), situated in the east of the Moroccan Mediterranean Sea. A comparison of allelic frequencies between populations allowed a first estimation of their genetic homogeneity. In fact, Allele frequencies for two loci didn't show variation between populations (Tab. II). The output of LOSITAN clearly indicates that no outlier position of *LDH** and *PGM** loci (Fig. 2); no polymorphic loci deviates from the expected under neutrality.

Table I. - List of samples and genetic variation data within each sampling site. Hobs = observed heterozygosity, He = expected heterozygosity, F_{IS} = Wright's fixation index.

Sampling site	Coordinates	Sample size	He	Hobs.	F_{IS}	p-value
1004	N35°08'24", W02°00'00"	20	0.196	0.040	0.805	0.0000
1009	N35°24'36", W02°48'00"	30	0.180	0.107	0.422	0.0023
1015	N35°14'24", W03°50'24"	30	0.196	0.120	0.402	0.0035
2005	N35°19'12", W06°13'12"	50	0.195	0.136	0.311	0.0111
2025	N33°28'48", W08°21'00"	47	0.190	0.094	0.516	0.0000
3032	N30°22'48", W09°43'12"	50	0.194	0.058	0.706	High. sign.
4006	N25°45'00", W14°41'24"	50	0.197	0.108	0.461	0.0000
4038	N24°19'12", W15°54'00"	31	0.189	0.142	0.265	0.1007
4058	N22°46'12", W16°30'36"	35	0.197	0.074	0.632	High. sign.
4065	N22°16'48", W16°48'36"	50	0.188	0.084	0.561	High. sign.
4068	N21°53'24", W17°02'24"	50	0.187	0.116	0.388	0.0009

The overall genetic differentiation was low, $F_{ST} = 0.00770$, reflecting no structuration between populations. The test of genetic differentiation among all pairs of samples illustrates allele frequencies similarity. F_{ST} estimates ranged from -0.03370 to 0.06888 (Tab. III). These values are a sign of a low degree of genetic divergence between samples. Consequently these populations could be considered to be homogenous. UPGMA phenogram (Fig. 3) based on genetic distances and using a non-biased algorithm of (Nei, 1978), allows to highlight the important genetic proximities between samples.

The hierarchical AMOVAs, performed using five geographic subdivisions, supported global genetic homogeneity among

Table II. - Allele frequencies and gene diversity for each sample of *E. encrasicolus*. n: sample size; Ho: observed heterozygosity; He: expected heterozygosity; F_{IS}: Wright's fixation index.

Locus	Allele	Sampling site										
		4068	4065	4058	4038	4006	3032	2025	2005	1015	1009	1004
*LDH*1*												
n		50	50	35	31	50	50	47	50	30	30	20
	75	0.6	0.52	0.486	0.661	0.58	0.46	0.649	0.58	0.6	0.717	0.6
	100	0.4	0.48	0.514	0.339	0.42	0.54	0.351	0.42	0.4	0.283	0.4
He		0.480	0.499	0.500	0.448	0.487	0.497	0.456	0.487	0.480	0.406	0.480
Ho		0.320	0.280	0.229	0.419	0.320	0.200	0.192	0.320	0.400	0.367	0.100
*PGM*1*												
n		50	50	35	31	50	44	47	50	30	30	20
	80	0.35	0.33	0.414	0.468	0.51	0.386	0.457	0.58	0.5	0.55	0.5
	100	0.65	0.67	0.586	0.532	0.49	0.614	0.543	0.42	0.5	0.45	0.5
He		0.455	0.442	0.485	0.498	0.500	0.474	0.496	0.487	0.500	0.495	0.500
Ho		0.260	0.140	0.143	0.290	0.220	0.091	0.277	0.360	0.200	0.167	0.100
Per sample over all loci												
He		0.187	0.188	0.197	0.189	0.197	0.194	0.190	0.195	0.196	0.180	0.196
Ho		0.116	0.084	0.074	0.142	0.108	0.058	0.094	0.136	0.120	0.107	0.040
F_{IS}		0.388	0.561	0.632	0.265	0.461	0.706	0.516	0.311	0.402	0.422	0.805
		0.0009	High. sign.	High. sign.	0.1007	0	High. sign.	0	0.0111	0.0035	0.0023	0

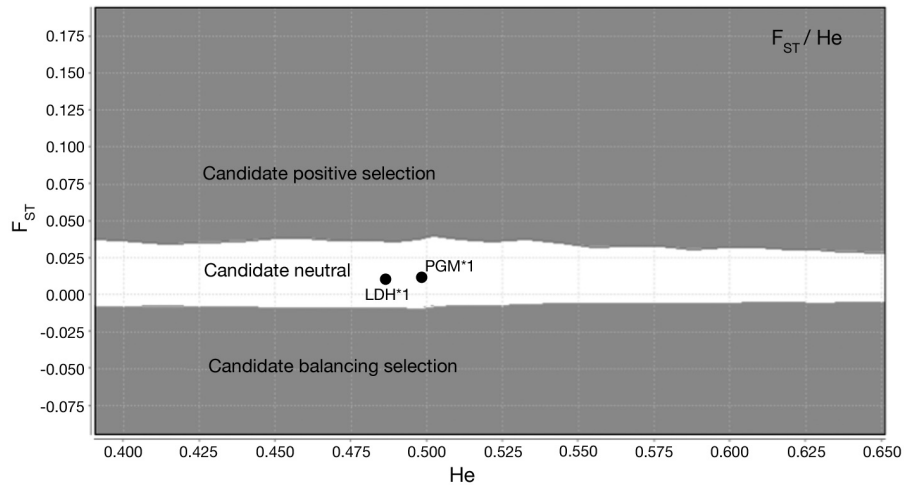

Figure 2. - Beaumont & Nichols FDist neutrality test performed using LOSITAN.

Table III. – Wright's standardized variance in allele frequency (F_{ST}) values for each pair of populations using the theta (θ) estimation of Weir and Cockerham (1984).

Station	4065	4058	4038	4006	3032	2025	2005	1015	1009	1004
4068	-0.00767	-0.00044	0.00093	0.01226	0.00560	-0.00042	0.03993	0.00462	0.03701	-0.00327
4065	–	-0.01097	0.02118	0.02122	-0.01002	0.01796	0.05094	0.01598	0.06725	0.00755
4058	–	–	0.01168	-0.00062	-0.01986	0.00933	0.01861	-0.00335	0.04781	-0.01336
4038	–	–	–	-0.00973	0.02669	-0.01905	0.00271	-0.01729	-0.01171	-0.02634
4006	–	–	–	–	0.01327	-0.00778	-0.00902	-0.01904	0.00155	-0.02755
3032	–	–	–	–	–	0.02386	0.03567	0.01077	0.06888	0.00153
2025	–	–	–	–	–	–	0.00564	-0.01605	-0.00642	-0.02494
2005	–	–	–	–	–	–	–	-0.01122	0.00231	-0.01900
1015	–	–	–	–	–	–	–	–	-0.00685	-0.03370
1009	–	–	–	–	–	–	–	–	–	-0.01656
1004	–	–	–	–	–	–	–	–	–	–

groups (-0.00226 < Fct < 0.01221) (Tab. IV). This result was concordant with the results of the test of genetic differentiation. From this analysis, we could deduce that the low genetic differentiation observed is not necessarily due to a sampling artifact (Whalund effect). Indeed, the difference between groups (Fct) and the differences between populations within the same group (Fsc) are low and not significant (Tab. IV).

The results of gene flow estimates obtained for the anchovy confirmed the statements above. It seems to reflect a high degree of connectivity between most of the populations analysed in this study, except for the populations of Mediterranean Sea and the south Atlantic of Morocco, which have nevertheless a limited gene flow (Tab. IV).

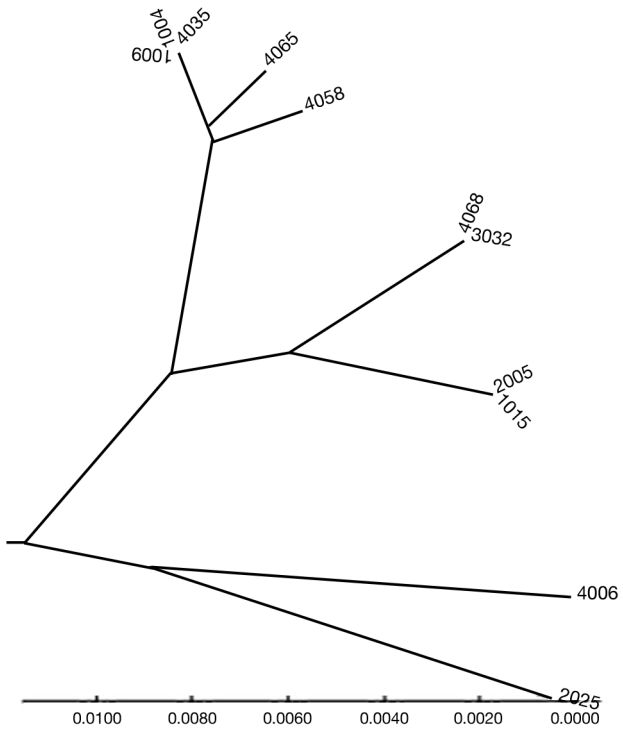

Figure 3. - UPGMA phenogram based on distance matrix of Nei (1978) showing the relationships between samples.

DISCUSSION

This study, based on the electrophoresis of allozymes used since 1966 (Buth, 1984; Pasteur *et al*., 1987), derives its reliability from the large number of samples and individuals analysed along the Moroccan coats (over a length of 2387 km). The limits concerned essentially the limitation of observed polymorphic loci found among the 11 enzyme systems tested. The samples were collected during the European anchovy spawning period, preventing thus a mixing between populations.

Significant heterozygote deficits are likewise an important result arising from this study (overall $F_{IS} = 0.48959$, $p < 0.001$). Except for Dakhla population (4038), all populations are not in Hardy-Weinberg equilibrium "HWE" (Tab. I). This result is in accordance with that obtained by Magoulas *et al*. (2006), Borrell (2012) and Silva *et al*. (2014) on anchovy populations across Atlantic Ocean and Mediterranean Sea. Heterozygote deficiency could be due to laboratory or sampling artefacts (i.e., null alleles or Wahlund effects) or biologic factors (i.e., consanguinity, selection,…) (Nei, 1987). In our case, heterozygote deficiency could not be explained on the basis of laboratory artifact (i.e., null alleles) because for two loci tested, we did not observe plainly any null homozygous among the analysed individuals. This could be the result of negative selection, refuting that individual presenting "null homozygous" dies in the nature; so, we cannot observe this pattern in the laboratory. Theoretically, the Wahlund effect could result either from simple mixing of animals from the two entities (mixed stock hypothesis), or from the existence of recent hybridization, or any combination of these phenomena in various proportions between samples (Oueslati *et al*., 2014). Previously, the hypothesis of the existence of a new inshore anchovy species, *E. albidus*, which is less common than the oceanic species *E. encrasicolus,* was suggested as an explanation of this heterozygote deficiency by Borsa (2002), Borsa *et al*. (2004) and Magoulas *et al*. (2006). It is difficult for us to correlate the results of the present study with the existence of *E. albidus*, since we did not analyse any sample from the lagoon. However, the test for selection (Beaumont and Nichols, 1996; Fig. 2) did not reveal any particular trends in polymorphic loci, refuting the idea that the loci were under selection or balancing selec-

Table IV. - AMOVAs analysis comparing two groups separated by a barrier or a long distance. Med/ATL: group 1 included samples from 1015, 1009 and 1004 / group 2 included samples from 4068, 4065, 4058, 4038, 4006, 3032, 2025 and 2005. Med and ATL-N/ATL-S: group 1 included samples from 1015, 1009, 1004, 3032, 2025 and 2005 / group 2 included samples from 4068, 4065, 4058, 4038 and 4006. Med/ATL-N: group 1 included samples from 1015, 1009 and 1004 / group 2 included samples from 3032, 2025 and 2005. Med/ATL-S: group 1 included samples from 1015, 1009 and 1004 / group 2 included samples from 4068, 4065, 4058, 4038 and 4006. ATL-N/ATL-S: group 1 included samples from 3032, 2025 and 2005 / group 2 included samples from 4068, 4065, 4058, 4038 and 4006.

Groups / Source of variation	Med /ATL	Med and ATL-N /ATL-S	Med /ATL-N	Med /ATL-S	ATL-N/ATL-N
Among groups Fct	0.00671**	0.00314***	-0.00106	0.01221*	-0.00226*
Among populations within groups Fsc	0.01083***	0.01130***	0.01339***	0.00580***	0.01503***

tion; the result of this test (the two loci evolve under the neutrality) could correspond to hybridization between two ecotypes. This hypothesis will be tested in our future work by analysing samples from lagoon and from the open sea using microsatellites marker.

Inbreeding is likely another hypothesis to explain the observed HWE departure. However, it is difficult to verify this hypothesis on a pelagic species such as anchovy, which is characterized by a large effective population size, high fecundity, a long spawning period and an external fertilization associated with a strong dispersion off eggs and larvae (Belvèze, 1984; Bakun, 1996; Fréon and Misund, 1999; Schwartzlose *et al*., 1999; Berraho, 2007). Nevertheless, all these species features reduce the likelihood of inbreeding in anchovy.

The main result of this study is that the level of differentiation among populations is overall low along the Moroccan coasts. Our samples were collected in general from open oceanic areas (depth between 20 and 60 m), on board of the R/V *Amir Moulay Abdallah*. We neither included samples from inshore (less than 20 m deep), nor from lagoon because fishing in those areas is not allowed in Morocco. Despite the limited number of polymorphic loci, our results were consistent with previous studies, which showed similarity or homogeneous main clusters among open sea populations (Bembo *et al*., 1996b; Tudella *et al*., 1999; Borsa *et al*., 2004; Sánchez-Velasco *et al*., 2006; Magoulas *et al*., 2006; Bouchenak-Khelladi *et al*., 2008; Zarraonaindia *et al*., 2009, 2012; Borrell *et al*., 2012; Oueslati *et al*., 2014; Silva *et al*., 2014). The lack of significant differentiation between populations, even with the potential presence of two anchovy's ecotypes, considers a complex differentiation model. The balance between gene flow and genetic drift determine the differentiation level and the convergence speed to this equilibrium. In general, the genetic differentiation process is counterbalanced by homogenizing force of gene flow. The low level of genetic differentiation might imply a strong relationship between coastal and offshore anchovy, and indicate that most part of the genome remains sparsely differentiated. However, this seems to agree with the model of semi-permeable barrier (Wu, 2001), which predicts a free dissemination of alleles between species in divergence since the loci involved in reproductive isolation are not enough to reduce gene flow across the genome (Barton and Bengtsson, 1986; Feder *et al*., 2012). Furthermore, Bierne *et al*. (2003) have showed that genetic differentiation across the hybrid zone varies greatly among allozyme loci, and that some allozyme loci show minimal population structure, similar to nDNA loci. In addition, no significant genetic structure was observed among offshore populations in the Alboran Sea and among oceanic populations in the Atlantic from Portugal to Senegal (Tudela *et al*., 1999; Magoulas *et al*., 2006; Borrell *et al*., 2012; Zarradonia *et al*., 2012; Oueslati *et al*., 2014; Silva *et al*., 2014). With regards to our analysed samples, we rather expected high level of genetic differentiation, especially on the Atlantic coast due to the potential presence in the area of hydrological barrier that could limit gene flow. In fact, the population richness of a species (number of autonomous populations) depends on the number of physical structures present within its distribution area, to which early adaptation encourages retention (Sinclair, 1988; Sinclair and Iles, 1989).

Barriers of the gene flow have been suggested for other species between north and south Moroccan Atlantic Ocean such as in the Ghir Cape with a hydrographic front (in the Bay of Agadir) for *Sardina pilchardus* (Chlaida *et al*., 2005, 2008; Atarhouch *et al*., 2007) and for the mussel *Mytilus galloprovincialis* (Jaziri and Benazzou, 2002). These barriers are the result of an intensive hydrological activity related to the Canaries current and the upwelling phenomenon that characterize the Moroccan coast (Le Floch, 1974; Belvèze and Erzini, 1983). However, some barriers can be functional for some species and not necessarily for others (Borsa, 2010). We think this is the case in our study.

Several authors have already described the hydrodynamic environment in this region. They confirmed the seasonal pattern of hydrological conditions reigning along the Moroccan coast and reported that the Canary Current and upwelling activity is more intense during summer (Arıistegui *et al*., 1994; Barton *et al*., 1998, 2004; Stevens and Johnson, 2003; Pelegrí *et al*., 2005). Indeed, meso-scale activity in the Canary Current Upwelling System (CCUS) is complicated (CCUS covers the latitudinal range 12-43ºN). A succession of jets, meanders, eddies, upwelling filaments, coastal counter-currents and buoyant plumes that represent the "weather" of the ocean, largely governs the ecosystem functioning. This meso-scale activity is a fundamental scale for the marine ecosystem behaviour, because the spatial and temporal scales of importance for marine plankton communities are mainly related to meso-scale features (ICES CM, 2012). It was also discussed that coastal upwelling and filaments are responsible mechanisms of transport and retention for neritic ichthyoplankton in the CCUS (Arístegui *et al*., 1994; Rodríguez *et al*., 1999, 2004).

On the Moroccan shelves, opposite to the sardine (*Sardina pilchardus*), which have a wintering spawning period, anchovy spawns mainly in summer (July and August) (Berraho, 2007). In this period, hydrological barrier, completely onset in winter for sardine (Chlaida *et al*., 2008), is permeable for anchovy in summer because of the shift in the hydrodynamic environment. Johnson and Stevens (2000) and Stevens and Johnson (2003) explained that difference in vertical circulation, depending on the season in the CCUS, induces changes in physical factors, which would become favourable to mixture between genitors and larval dispersal during anchovy spawning period. Indeed, a larval exchange is

assumed for most species to be the major mechanism uniting spatially discrete population (Planes *et al.,* 1998). The general lack of barriers in marine waters can facilitate high levels of contemporary gene flow between populations, especially in species with pelagic eggs or larval phase, or in species with highly migratory adults (Magoulas *et al.,* 2006). On the other hand, our data showed an important gene flow between populations (Tab. IV). Borsa *et al*. (2004) confirm that the lack of detectable genetic differences across vast distances (several thousand km) within a group implies high levels of intragroup gene flow.

CONCLUSION

The present study, based on enzymes electrophoresis, despite the limited number of polymorphic loci, sheds light on genetic structure of anchovy populations along the Moroccan coast. The analysed samples were collected among oceanic populations, which form the main area of fishing vessels activity. No significant genetic differentiation was observed among these populations. On another hand, our results support the hypothesis of the hybridization between two lineages (two species), the analysis of other polymorphic loci for anchovy should permit the confirmation of these results. Future research should then focus on microsatellites markers analysis and finer sampling of oceanic and inshore anchovies, in order to provide further details concerning population genetics of *E. encrasicolus* along the Moroccan coast.

Acknowledgements. – We wish to thank F. Bonhomme for his helpful advice and guidance; H. Chihabi for her laboratory contribution to this work; N. Charouki and S. Ayoubi for providing the samples; Naoki Tojo and JICA/INRH project for their support, and referees for their constructive remarks and comments. This research was supported by the INRH and Faculté des Sciences de Rabat. The analysis techniques used in this work are in compliance with the legislation in use in Morocco.

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